

Directed migration of circulating polymorphonuclear leucocytes in patients with rheumatoid arthritis: a defect in the plasma

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SUMMARY The migration of peripheral polymorphonuclear leucocytes (PMNs) of patients with rheumatoid arthritis has been studied both *in vivo* and *in vitro*. A significant reduction in the accumulation of PMNs in skin chambers in patients with rheumatoid arthritis compared to controls was observed but no defect in cell movement was detected when the isolated PMNs from the patients were exposed to activated control plasma. However, when PMNs from the control group were tested against activated plasma from patients with rheumatoid arthritis there was a significant decrease in their chemotactic response. It is proposed that there is a humoral defect in the plasma of the patients.

Abnormalities of polymorphonuclear leucocyte (PMN) migration have been described in many diseases (Quie and Cates, 1977). Evidence has been presented for a defect in the *in vitro* chemotactic responsiveness of the cells in patients with rheumatoid arthritis (Mowat and Baum, 1971). It was suggested that ingestion of immune complexes may be a possible explanation for the reduced responsiveness of the PMNs to chemotaxins. More recent work with patients with rheumatoid arthritis (Roberts-Thomson *et al.*, 1976) and Felty's syndrome (Hurd *et al.*, 1977) has lent support to the proposal. Other workers (Goetzel, 1976), however, have been unable either to confirm the abnormal movement *in vitro* by PMNs from rheumatoid patients reported in the earlier studies or to demonstrate an abnormality in the accumulation of PMNs into skin chambers in similar patients (Segal and Loewi, 1976).

In this communication we describe the findings from 2 separate series of experiments which were designed to re-examine the chemotactic response of PMNs in rheumatoid arthritis both *in vivo*, using skin chambers to observe the accumulation of PMNs into autologous serum, and *in vitro* using modified Boyden chambers to measure the directed migration of cells in both rheumatoid and control groups. The experiments were designed to determine if a defect in cell movement existed in patients with

rheumatoid arthritis and, if so, whether the defect was associated with either the leucocytes themselves or with some abnormality of the plasma.

Patients and methods

All the patients reported on in these studies were attending rheumatology outpatient clinics at King's College Hospital and had definite or classical rheumatoid arthritis (American Rheumatism Association criteria).

In the *in vitro* studies 28 patients constituted the rheumatoid group (12 male and 16 female; average age 58.3 years, range 34–76) with a mean duration of disease of 7 years (range 1–19 years). These patients were on a variety of drug treatments including gold, penicillamine, and steroids, and the majority were also taking a nonsteroidal anti-inflammatory drug such as indomethacin or salicylate. The control group consisted of 23 patients (9 male and 14 female; average age 51 years, range 23–76 years) suffering from a variety of non-inflammatory diseases such as low back pain and osteoarthritis. The majority of these patients were not receiving any drug therapy.

The *in vivo* studies were performed on 17 patients with rheumatoid arthritis (4 male and 13 female; average age 60.1 years, range 49–76 years) with a mean duration of disease of 11.8 years (range 1–35 years). These patients were all being treated with nonsteroidal anti-inflammatory drugs. In addition 1

patient was on gold treatment and 16 were on D-penicillamine or fenclofenac or placebo as part of a double blind clinical trial. The control group consisted of 2 sub groups—one of 5 young healthy laboratory workers (average age 24 years, range 21–27 years), and the other of 11 older patients with osteoarthritis (average age 53.1 years, range 43–64 years). Two of the patients in this second group were receiving nonsteroidal anti-inflammatory drugs.

IN VITRO PROCEDURES

Samples (20 ml) of blood were obtained by venepuncture and transferred to bottles containing 200 units of preservative-free heparin. 5 ml of each sample was centrifuged (15 min at 2000 *g*), the plasma removed, and samples used as described below either immediately or after storage at -20°C for no longer than 2 weeks. Cell populations were obtained from the remaining blood by dextran sedimentation as described previously (Walker *et al.*, 1975). All cell populations (75–80% PMNs) were resuspended to give a concentration of between 2.0 and 2.5 $\times 10^6$ cells ml^{-1} in Medium 199 buffered to pH 7.3 with HEPES (25 mM). Chemotaxins were generated in the plasma samples by incubation with zymosan (2 mg ml^{-1}) for 30 min at 37°C followed by heat inactivation for 30 min at 56°C . The zymosan was removed by centrifugation and the activated plasmas were diluted 1 in 10 with Medium 199. Directed migration was measured in modified Boyden chambers (Neuroprobe, Inc., Maryland, USA) fitted with 5 μm Millipore filters. The cell suspension (0.5 ml) was placed in the upper chamber and 1.5 ml of the diluted plasma into the lower chamber, and each experiment was performed in duplicate. The chambers were incubated at 37°C for 2 h, after which the filters were removed, fixed, and stained. The number of cells that had reached the lower surface of the filter was counted in 5 separate high-power fields under $400\times$ magnification. α_1 -Antitrypsin, α_2 -macroglobulin, and total C3 and C4 concentrations in the plasma of the rheumatoid patients were determined with commercially available immunodiffusion plates (Hoechst Pharmaceuticals). Functional factor B and total alternative pathway capacity were measured by means of bystander haemolysis of guinea-pig erythrocytes suspended in agarose as the indicator system (Martin *et al.*, 1976). Measurement of other plasma proteins such as immune complexes or cryoglobulins was not carried out.

IN VIVO PROCEDURES

A modification of the skin chamber technique described by Senn *et al.*, (1969) was used. Blood was

taken from each individual immediately before the experiment from which cell-free serum was obtained. After prehydration of the skin with pads soaked in normal saline the skin over the flexor aspect of the forearm was cleaned with 70% isopropyl alcohol. Two small abrasions were made by means of an electric drill with a spherical dental burr (0.6 cm diameter). The mean area of the abrasions was $0.080 \pm \text{SEM } 0.005 \text{ cm}^2$, $n=50$. Occasional small bleeding points were seen, but bleeding stopped within 3 minutes. Plastic skin chambers (2.5 ml capacity) were placed over the abrasions and were held in place by a silicone adhesive (Medical Adhesive B, Dow Corning Corporation) and surgical tape. Autologous serum (2 ml) was introduced into each chamber through a small hole which was plugged. The chambers were kept on the arm for 24 h, after which the serum was aspirated and placed into plastic tubes. The area of each abrasion was measured with a Vernier caliper after removal of the skin chamber. The number of leucocytes in the serum was determined by standard methods and the number of cells migrating into 1 ml of serum per cm^2 in 24 h was calculated. Over 95% of the migrating cells were PMNs.

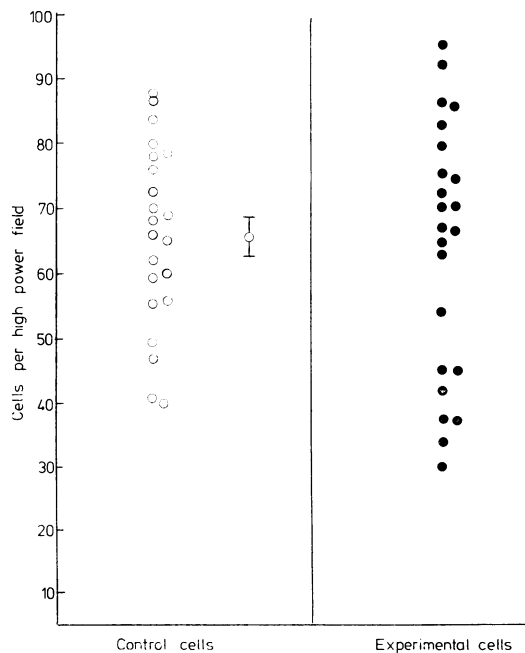


Fig. 1 Response of polymorphonuclear leucocytes from control and experimental groups to activated plasma from control group. \circ PMNs from control group. \bullet PMNs from experimental group. \bar{x} Mean = standard error.

Results

IN VITRO

The results in Fig. 1 show that there was no significant difference in the directed migration of the peripheral leucocytes prepared from either group of patients when the cells were exposed to activated control plasma in vitro. In contrast, the results in Fig. 2 show clearly that leucocytes prepared from control patients showed a significantly diminished response when exposed to activated rheumatoid plasma (41% reduction, $P < 0.001$, Student's *t* test) when compared to activated control plasma. The response of control cells to control activated plasma did not vary more than 10% from day to day. This reduced chemotactic attraction bore no relation to other parameters measured, that is, duration and severity of the rheumatoid arthritis, erythrocyte sedimentation rate (ESR), the presence of rheumatoid factor, α_1 -antitrypsin and α_2 -macroglobulin concentrations nor to drug therapy. No deficiencies in the patients' plasma as regards the total concentrations of the complement components C3 and C4, the functional activity of Factor B, and the functional capacity of the alternative pathway were observed.

IN VIVO

Fig. 3 illustrates the results obtained with the

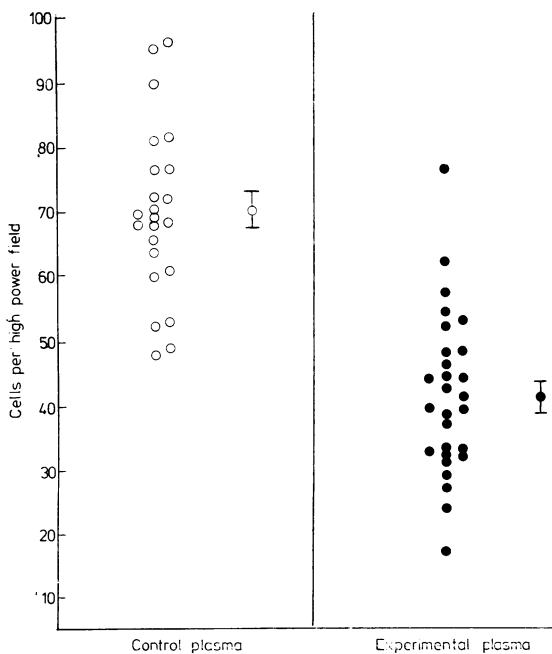


Fig. 2 Response of control polymorphonuclear leucocytes to individual activated plasma samples from control and experimental groups. Symbols as for Fig. 1

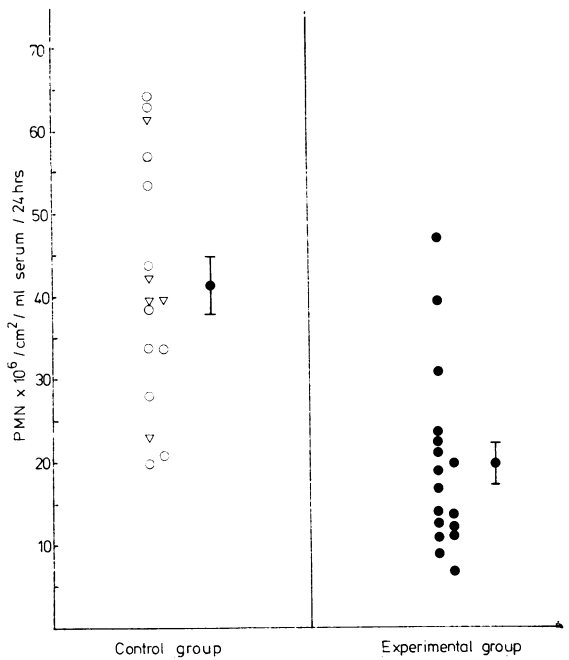


Fig. 3 Comparison of PMN accumulation in skin chambers between control and rheumatoid patients. \circ Age-matched controls. ∇ 'Young' control group. \bullet Experimental group. \bar{x} Mean = standard error

control subgroups compared with those obtained with the rheumatoid patients. There was no difference in cell accumulation between the two control subgroups, but there was a significant reduction in PMN accumulation in the patients with rheumatoid arthritis (46.5%, $P < 0.001$). This diminished cellular responsiveness in vivo was again unrelated to other parameters such as ESR, articular index, duration of disease, the patient's age, or to drug therapy.

Discussion

Various defects have been reported, either cellular or humoral, in several chronic inflammatory diseases including rheumatoid arthritis (Mowat and Baum, 1971), systemic lupus erythematosus (Clark *et al.*, 1974; Goetzl, 1976) sarcoidosis (Maderazo *et al.*, 1976; Gange *et al.*, 1977) and Crohn's disease (Segal and Loewi, 1976).

The observation in the present paper of a diminished accumulation of PMNs in skin chambers in rheumatoid arthritis patients is contrary to other findings (Segal and Loewi, 1976) but reflects our in vitro observations. The accumulation of PMNs in

vivo is the end product of a variety of effects such as directed and stimulated random migration, vascular status, blood flow, and the presence or absence of serum inhibitors. It is thus impossible to distinguish from the observations in vivo which of these many factors may be responsible for the depressed cellular response. However, none of the patients or controls in this or the subsequent study showed any indications of major differences in vascular status such as Raynaud's disease. Although the in vitro experiments were performed with cells and plasma samples obtained from a different series of patients, it has been possible to demonstrate that, while PMNs obtained from these patients respond normally when exposed to activated control plasma, there is a factor or factors present in their plasma which in some way depresses the chemotactic response of normal PMNs when exposed to their activated plasma in modified Boyden chambers.

Inhibition of the release of chemotaxins during complement activation by zymosan is unlikely to be a mode of action, since the measurement of alternative pathway capacity which utilised whole plasma was unaffected. A further mode of action of the plasma material may be the deactivation of chemotaxins once they have been generated from the parent complement molecules. Such materials have been described in a variety of diseases (Ward *et al.*, 1977; Van Epps and Williams, 1978). Using an agarose plate chemotactic assay we have found that control PMNs migrate normally towards activated rheumatoid plasma (results not shown). The apparent discrepancy between the 2 methods indicates, firstly, that the factor(s) is exerting its inhibitory action directly on the cell (Goetzl, 1975), and, secondly, the material is of considerably higher molecular weight than the complement-derived chemotactic peptides. Experiments to confirm the mode of action and to clarify the physical properties of the inhibitory materials are in progress.

A possible criticism of these results is that the drug therapies employed may exert an effect on the in vivo cell accumulation or in vitro migration studies. While this cannot be totally ruled out, there is no apparent correlation between the drug treatment of the patients who received skin chambers or the plasma activities of those patients used in the in vitro studies.

The presence of such an inhibitor raises a number of questions with respect to the disease. First, does it cause the propensity to infection observed in such patients? Secondly, is its presence a fundamental manifestation of the disease? A related and important query is whether diminished chemotactic responsiveness is a pathological reaction to the disease

process or whether it represents a normal defence mechanism. The present experiments have been concerned only with the behaviour of PMNs, and it is of some importance to extend the work to investigate whether the responsiveness of mononuclear cells, which are of more importance in chronic inflammation, is also affected.

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